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GENETIC APPROACHES TO SOMATIC CELL VARIATION: SUMMARY COMMENT

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Up to this point I would heartily concur with the felicitations that were offered to the organizers of this program, but in one respect I think they might have served us better. We have worked hard in crowded long sessions, and perhaps you deserve something more pleasant and jocular than I am competent to offer.

One thing I will not do is summarize what was said, since that will be found in the papers themselves. I suspect that my intended function is to summarize what was *not* said.

I left the last session before I was able to discover what the molecular basis of somatic variation is, and that was only so I would have an opportunity to collect these thoughts. Perhaps there will be some opportunity in the rebuttal to the discussion, or the discussion of the rebuttal, to make up for it.

I think we should be grateful to Dr. Stern for opening the session with his summary of very useful and important facts concerning processes of variation in somatic cells. An understanding of the mechanical history of the chromosome is, of course, basic to any of the further speculations that we may like to build, and it is some comfort, indeed, to know that there is rather concrete evidence for such processes as mitotic crossing over and endomitosis that we might like to invoke.

He also, I was glad to note, pointed out some difficulties. It is still rather hard to understand how precise triploid complements can be gotten by any simple somatic process. I think it may be necessary to give more attention to the problems of genome segregation that the late Professor C. L. Huskins, of Wisconsin, was so interested in, and which are paralleled in the enigma of the ciliate macronucleus.

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I am still rather puzzled why biologists show such a strong antisexual bias in the consideration of somatic cells. On the other hand, I think I would have been the first to ridicule the fantasy that viruses might carry bits of genetic material from one cell to another in a transductive process, and yet suggestions of this kind seem to be accepted with great gullibility. Projections for future experimentation on somatic cells have invoked transductive phenomena almost to the exclusion of mating. After all, if we combine Stern's discussion with Hauschka's, we will see that every single one of the unit processes needed for the technical handling of mating has been documented in somatic cells. True, they have not been serially documented on a given set of cells under experimental control. But we have reports of the fusion of somatic cells. We know that nuclei of binucleate cells can fuse, if only by coalescence of the spindles at the next mitosis. We know we can have somatic segregation as well as mitotic crossing over. Fifteen years ago we had a much more negative outlook with regard to the possibility of Mendelian analysis with such organisms as bacteria, viruses, and *Penicillium* than we now have for somatic cells.

Ephrussi initiated a provocative discussion on the classification of genetic phenomena, which is of some importance for any attempt to relate the facts of cellular differentiation to the framework of genetic theory. In the past, we have contrasted chromosomal versus extrachromosomal. Reporting on a recent conference on cytoplasmic heredity held at Gif, he suggests that this contrast may be misdirected. He would define hereditary phenomena as either "genetic" (in a stricter sense) or "epigenetic," according to whether the information is structural or based on some sort of dynamic flux equilibrium. In the English language, at least, this particular choice of terms may be confusing if only because "epigenetic" is already widely current in a different sense, e.g., in Waddington's book "The Epigenetics of Birds." The proposal is so provocative, however, that we ought not to dwell merely on its verbal aspects.

As has been pointed out, we can now begin to ask very concrete questions on the *chemical* basis of genetic differentials. We can no longer doubt the role of nucleic acids in this context, and rather than debate it any further I think we can **define** a category of genetic information as being "nucleic"; that is, depending on the *sequence of nucleotides* in a nucleic acid. By contrast, "epinucleic" information is expressed in another form, e.g., as an aspect of nucleic acid configuration other than nucleotide sequence or in polypeptide or polyamine adjuncts to the polynucleotide. We also have extranucleic information in molecules or reaction cycles not directly connected with nucleic acid. In accord with Ephrussi's suggestion, we might propose that nucleic information has the pervasiveness and static precision connoted by "genetic," whereas the epinucleic information regulates the manifestation of nucleic potentialities in the dynamic, temporally responsive functioning of actual development.

We can now resolve an earlier debate whether "self-reproducing particle" and "self-sustaining reaction cycle" are meaningfully different concepts (my own argument having been that the act of self-reproduction was just such a cycle). I must agree that there is at least this difference: the tremendous informational complexity of a long linear polymer that is replicated point by point in contrast to the one-bit, yes-or-no alternations we observe in the simpler feedback systems. In fact, the much relied upon criterion of mutability is another aspect of this complexity. A one-bit unit is either present or absent; mutation is the deletion or substitution of one bit in a long string.

At the present time, we know of no other biological system of replication or autocatalytic feedback (short of the whole organism!) that remotely approaches the informational complexity of a polynucleotide, which justifies the contention that the bulk of germinal genetic information is nucleic. On the other hand, we cannot readily visualize a mechanism for the determinate, systematic alteration of nucleotide sequences to account for cellular differentiation. For this reason, chromo-

somal nucleic variation, i.e., gene mutation, has been the least popular element of all genetic models of differentiation. This has left plasmagenes (extranuclear nucleic) and steady states (extranuclear epinucleic) as the chief contenders in the model-building Olympics. But there is now increasing concern for a hypothetical category sometimes overlooked: epinucleic variations of the chromosome. As Ephrussi pointed out, the genetic evidence for epinucleic variation in the nucleus is suppositious (and it cannot actually be quite compelling until we know the nucleotide sequence of a chromosome). However, in paramutation (a phenomenon I hope Dr. Brink will enlarge upon), in the differentiation of amphibian nuclei, and in *Salmonella* phase variation, we have a series of effects that lack the "molar indeterminacy" of ordinary gene mutation, enough to make us suspicious that they might be epinucleic. In the cytochemistry of chromosomes, we have rather more-direct evidence, e.g., in histone versus protamine in different nuclei and in the morphogenetic variation of localized bands of salivary-type nuclei in insects, but it is impossible to assess these qualities as features of cellular *heredity*. Epinucleic chromosome variation is therefore an entirely speculative hypothesis designed to leave some leeway for differentiation in the chromosome without having to invoke balky ideas of determinate changes in nucleotide sequences.

Obviously there is little to say about the details. Epinucleic variation might encompass dynamic equilibria, at chromosome loci, and involving genes and their products (ribonucleic acid protein?) along the same lines as parallel proposals for the cytoplasm. Furthermore, we should perhaps look for variations in nucleic structure that do not alter the fundamental sequence. The compact double helix is, of course, the idealized structure, on whose regular periodicity the X-ray diffraction diagram depends. The diagram cannot tell a great deal about the local deviations from the ideal structure, which may be most pertinent to the present dimension of genetic variation. It can say least of all, by present methods, on the detailed, transient structure of deoxyribonucleic acid (DNA) in the

metabolically active cell. The way in which proteins are coupled to DNA and their point-to-point specificity are open questions and may have much to do with local states. The coupling of polyamines has also been mentioned as another epinucleic variable. What is less clear is how such local states can be replicated along with the informational sequence. However, one can visualize that the accumulation of local specific products at an open region can help to dissociate compactly intertwined helices after replication and make them open as well. This notion supposes that the closed double helix is so compact, i.e., has so little residual chemical activity, that it cannot function without unravelling, either for self-replication or for its heterocatalytic function.

Genetic thinking about development has been dominated by the doctrine of genetic constancy: the conservation of genetic constitution in all the cells of an organism. The fact of differentiation immediately contradicts this generalization, and the paradox has raised a serious intellectual barrier between embryologists and geneticists. I suggest we now try out another *working hypothesis:* the conservation of nucleic information, or at least of chromosomal nucleic information in *normal* development (though we will doubtless have to make some concessions in special cases such as diminution in *Ascaris*).

One doctrine that was brought up in the last session, perhaps too often, was the pleonasm about doing embryology on embryos. My initial thought was to issue a recantation, but after the argument went on a little I began to think that maybe there was something in it after all, in the sense that the limiting factor in the analysis of differentiation is no longer the insufficiency of hypothetical models and partial analogies. Nucleic versus epinucleic and chromosomal versus extrachromosomal are each logically exhaustive classifications of all possible mechanisms, but the assessment of genetic approaches to somatic cell variation depends less on this intellectual exercise than on finding ways to ask critical questions

of embryos, as for example, R. Briggs and T. J. King have done.

Some of the selective methods that have to be devised to cope with populations of somatic cells are foreshadowed by the work of Cotterman and Atwood on the erythron. That there should be some technical difficulties at this stage of analysis is a predictable fact, but we can be certain that diligent concentration on these problems is going to provide some still unsuspected but indispensable tools for the detection and isolation of rare genotypes.

Klein's report and Mitchison's discussion represent the next important steps in the rational construction of a somatic cell genetics. Recombination between pure line cells, whether diploid, or worse if polyploid, will generate heterozygous cells. It will then be an immediate problem of technique to cope with heterozygotes, preferably by inducing segregation to uncover recessive markers. It is now clear that this can be done, though the mechanism still has to be worked out. On the basis of a straightforward tool for detecting rare segregation, we can look forward to development of techniques for inducing and controlling it — by analogy with the effects of ultraviolet light on heterozygotes of yeast, *Aspergillus,* and *Escherichia coli* and on heterokaryons of *Neurospora.*

The most puzzling feature of the results reported here is the asymmetry. Why should a tumor cell of constitution A/S give S/S homozygotes (or S hemizygotes) more often than A/A? Keep in mind that the hybrid was obtained from coisogenic lines that should not differ in much more than the H_2 locus itself. Klein reviewed a number of plausible explanations that further work will doubtless sort out. He left me the opportunity of adding another thought to his list. The very act of selection for a tumor introduces new factors into the cellular genotype. If one of the new mutations should be located on the chromosome carrying the H_2^s allele, only those segregants that continued to carry this chromosome would be detectable as rapidly growing tumors. To test this hypothesis it will be necessary to devise a selective method applicable to

non-neoplastic tissues as well. In the long run, just this sort of analysis will be needed to assay the various genetic bases of neoplasia.

Hauschka's and Ford's analyses of tumor cell populations remind us how much further the cytology of somatic cells has advanced than has their genetics. We can only begin to guess at the full implications of the karyotypic diversity in these populations, and this can only hint at the extent of variation of individual genes.

In the discussions much was said of the esthetic appeal, good manners, laboratory convenience, and karyotypic elegance of the Chinese hamster; regrettably we could not furnish a sample for your inspection at short notice. One assertion has been very puzzling to me, and it is one that could be broached only with a well-differentiated karyotype. This is the viability of *nullisomic* tumor cells. The Chinese hamster has the lowest chromosome number of its taxonomic section, which makes it difficult to invoke polyploidy. There are perhaps three points of view to consider: (1) that some mammalian complements include supernumerary, dispensable chromosomes, like the B chromosomes of maize. This solution might help to clear up the variability of chromosome number, 46, 47, 48, which Stern discussed for man; (2) that the nullisomic cells are the immediate progeny of irregular mitoses and have no capacity for indefinite survival; (3) that the phenogenetic functions of the various chromosomes are well ordered, so that one or more chromosomes simply do not have any genes that are indispensable to cellular viability in the protected environment of an ascites tumor. Such a chromosome is then supernumerary for the tumor cell, though it may be quite necessary for the normal development of the whole animal. The last suggestion has had little explicit support from what we knew of the distribution of gene functions; but it has been revived by the findings of physiologically correlated blocks of genes in bacteria.

Whatever our final conclusion proves to be, a nullisomic karyotype presents special problems of interpretation, apart

from those of interchromosomal balance presented by other types of aneuploidy.

In his account of remarkable studies on mutable genes affecting pericarp color in maize, Brink took pains to disavow their applicability to differentiation, chiefly because of the indeterminacy of the mutations. He was also careful to stress that he was not discussing McClintock's findings on mutable genes, from which she does construct a model. The essential features, as I understand her interpretation, are: (1) that the level of activity of any of a number of loci can be regulated by a transposable "controller"; (2) that these controllers are subject to "changes in local state"; and especially (3) that these changes of state occur not randomly but at sharply circumscribed intervals in developmental time. The last point is the kernel of McClintock's argument: if there are local changes in genes that occur at definite epochs of development, we have a chromosomal event, be it nucleic or epinucleic, that is tied to a developmental clock. The controllers that McClintock uses in her experiments would be pathological deviants (as they must, to be amenable to conventional genetic methods), but they still reflect the morphogenetic cycle of differentiated levels of activity of different genes. Unfortunately, the orderliness with which changes of state occur has not been reported in a detailed quantitative study, and we must rely on a few selected photographs and verbal accounts. Dr. Brink assured us that *his* mutable material did *not* show orderly patterns, and on this basis it is easy to see why these two investigators would draw different conclusions on the relevance of their material to developmental theories. I hope, however, that Dr. Brink will favor us with an account of another system he has been studying, the *R* locus, where he has evidence of orientation of genetic change by one allele acting on another.

The orderliness of normal development that impels us to invoke epinucleic parameters for its cellular genetic analysis stands in vivid contrast to neoplasia. It is therefore reasonably certain that we cannot insist on a unitary theory for the

initiation or progression of tumors. Development of a tumor represents the evolution of a cellular population, in which, in contrast to the fitness of the whole organism, adaptive fitness is reflected in the capacity for ever more rapid and unregulated growth. Thus any incident that initiates or promotes the cell's evolutionary progress to this end must be involved in carcinogenesis: the phenogenetic effect rather than the genetic mechanism tells what role it will play. Present knowledge places no bounds on the scope of mechanisms of variation that might contribute to a neoplastic phenotype, just as we do not attempt to account for the evolution of species by any single mode of genetic displacement. Indeed, it would be surprising if a neoplastic phenotype were always initiated by a single variational event, and we can suppose that the cumulation of several variations (whether by gene mutation, virus infection and transduction, plasmid segregation, recombinaation, or karyotypic upsets) will be necessary before a once normal clone transcends the threshold of malignancy. Once committed to this pathway, a "premalignant" cell may be expected to show accelerated evolution on account of its very augmentation of growth rate and its physiological unbalance. The experimentally designed bacterial populations that Braun displayed may be an introductory primer to the intricacies of the evolution of a tumor.

Before concluding, I would like to take the occasion for an appeal on behalf of the inbred mouse for sophisticated studies of somatic cell genetics. Inbred mice are, of course, quite indispensable for transplantation work, but they have been relatively unpopular in tissue culture. There is no doubt of the anthropocentric glamour of using human tissues, nor can one ignore the investment that has gone into the development of HeLa cells as nearly standard material, and it would be impossible to discount the splendid progress that has been made with it. But when we approach questions of genetic analysis, the unpredictable genetic constitution of HeLa cells, their irreversible heteroploidy and heterozygosity, and the lack of a defined compatible host for retransplantation are likely to

lead to treacherous blind alleys. The type of study that Klein has been doing illustrates the opportunities that await the systematic use of pure line material in tissue culture, which include the facility of constructing known genotypes by conventional mating.

The limitations of human tissues are especially evident when the occasion arises to test cultures *in vivo,* in retransplantation to a fully compatible host. We have now, for example, a confused and contentious picture of the incidence of neoplastic transformation in normal tissues maintained in culture and can never hope to reach a definite conclusion with the use of human material.

OPEN DISCUSSION

AUERBACH [2]: I do not quite see why nullisomics among tumor cells must be a problem. Tumor cells are parasitic cells fed by the host, and even in tissue cultures there may be close cooperation between cells. I know of at least two cases where loss of genetic material could be tolerated by cells in close contact with similar cells. One is in the tapetum of a plant — I forget which — studied by Barber and Callan; the other, the synchronized postmeiotic divisions of the male germ cells of the louse that G. Pontecorvo studied. In the latter case, very large chunks of genetic material could be lost without ill effect on the cell.

STERN [3]: Dr. Lederberg was very much in favor of hybridization of somatic cells, and I would like to give an example where this perhaps has been accomplished. The German botanist, Hans Winkler ('38), made graft hybrids between the two species *Solanum nigrum* and *S. lycopersicum.* In the great majority of cases he obtained chimeras, certain layers of cells being derived from *S. nigrum* and others from *S. lycopersicum.* But after many years of work, he thought he also had two cases to which the term "Burdonen" could be

[2] Charlotte Auerbach, Oak Ridge National Laboratory; on leave from University of Edinburgh.

[3] Curt Stern, University of California, Berkeley.

applied, defined as true vegetative hybrids resulting from nuclear fusion of cells from scion and graft. Only the epidermis of the two plants had Burdo character as indicated by (abnormal!) chromosome counts and phenotype.

I would prefer not to call this sexuality, even though the whole process was there. It seems to me that we perhaps speak of sexuality best when two cells are "made for each other"; in Winkler's cases, it was "just happening."

My other comment is related to Dr. Lederberg's reference to McClintock's finding that there was a specific time in development when the changes in status occurred. She stressed that they were tied to the developmental clock. In *Drosophila*, somatic crossing over is also somewhat tied to time and place of development. Sizes and frequencies of spots vary in various body regions. Even the location of the place of somatic crossing over is correlated with the developmental pattern. Nevertheless, I do not regard this correlation as furnishing a model for genic control of differentiation.

STREHLER[4]: Most of the mechanisms that have been suggested here for genesis of tumors seem to me to have first-order characteristics; that is, you would expect these events (or accidents) to accumulate more or less at a constant rate with respect to time. Yet, the probability that individuals in a population will die of tumors increases exponentially. Is there a hypothesis or an explanation that would logically relate these two phenomena?

KOLLER[5]: I believe it was Mahler who, a long time ago when looking at the increasing incidence of cancer with age in humans, suggested that there must be more than one event in the cell for the transformation of the cell, and he calculated five or six. Following Mahler's suggestion, after a few years, Northerly, from Sweden — who was not a biologist but I believe an engineer who took a fancy to this problem and made an investigation — came to the same conclusion. The problem was then discussed in the British Journal of Cancer by Alde-

[4] B. L. Strehler, National Institutes of Health, Baltimore City Hospitals.
[5] P. C. Koller, Chester Beatty Research Institute.

It may be argued that endocrine tumors are exceptional. They do not seem to be exceptional to me in any other respect but that, with regard to the systems where they arise, we know something about the growth controlling homeostatic influences whereas we usually know nothing about the other systems.

I should like to add just one more point to the plea Dr. Lederberg made with regard to suitable material for the study of somatic variation and of carcinogenesis, with which I fully agree. I feel that one set of markers that ought to be included in such studies should be of a nature related to the essential phenomenon in malignancy. Hormone dependence of endocrine organs and tumors would be a major candidate at the present stage.

STREHLER: Apropos of Dr. Klein's remark! One would certainly have to agree about the heterogeneity in the human population in which the incidence of tumors is probably best documented. Nevertheless, there is one study by Simms and Berg in which they measured the incidence of tumors quite accurately for a single kind of tumor (in a highly inbred strain of rats) and still found an approximately exponential increase with age. One robin does not make a spring, but I do not think that one can dismiss the extremely elegant fit between log probability of tumor formation and age by simply saying that there is great heterogeneity in the biological material. That may be relevant or it may not be. It may be that there is something about the mechanism of tumor genesis or a change in the organism's resistance, as Dr. Auerbach and others have suggested, that produces this particular exponential kinetics.

STRAUSS[7]: I still wonder whether it is necessary to accept the idea that the polynucleotide base sequence in DNA actually determines gene action. If amino acid sequence determines function, proteins with different sequences should have different functions, and the reverse should also be true. However,

[7] B. S. Strauss, Syracuse University.

there are different amino acid sequences in insulins from a number of species, but these proteins have the same function. Furthermore, separate investigations by Gladner, Schaffer, F. J. Dixon, H. Neurath, D. E. Koshland, and others seem to indicate an identical amino acid sequence at the active site of the enzymes thrombin, chymotrypsin, trypsin, and phosphoglucomutase. All these enzymes have very different specificities. It seems to me that the amino acid sequence in proteins may perfectly well be determined by the sequence of bases in DNA and that this is the source of the species specificity of proteins. But I think that protein activity may be determined by a three-dimensional structure superimposed upon the base order.

BRINK[8]: Since Dr. Lederberg made brief reference to it, members of the group might be interested if I said a little more about the curious kind of heritable change that we recently observed at the *R* locus, conditioning aleurone and plant color in maize. The meaning of the evidence for the problem of differentiation is unknown, but the extraordinary lability observed at the locus conceivably is significant in this connection.

When pollen of our standard *RR* strain (self-colored aleurone) is used on *rr* plants (colorless aleurone), the resulting kernels are darkly mottled (*Rrr*). In a strain having the same highly inbred background the mating, *rr* ♀ × R^{st} R^{st} ♂ (stippled aleurone) yields stippled kernels ($R^{st}rr$). It would be expected, therefore, that when pollen from the heterozygote, RR^{st}, is used on *rr* individuals, one-half the kernels would be darkly mottled and one-half would be stippled. This, however, is not what is observed. The stippled class of kernels is regularly formed, but the other class of kernels expected (dark mottled) does not appear. In place of the latter is a new phenotype (*R'rr*) characterized by weakly pigmented aleurone. Plants grown from the associated *R'r* embryos transmit the *R'* allele regularly, although the phenotype of the offspring is shifted somewhat toward that of standard *Rrr* kernels.

[8] R. A. Brink, University of Wisconsin.

Complete reversion of R' to R, however, has not yet been observed.

The change in determinative action of R to the R' level is unique in several respects. (1) It occurs invariably in RR^{st} heterozygotes and not sporadically as is characteristic of mutation; (2) the genetic change is directed rather than random; and (3) the alteration of standard R to R' is partially reversible, likewise with complete regularity. The experiments also show that in heterozygotes with marbled (R^{mb}), an allele distinct from stippled, standard R invariably changes to a third form, which may be designated R'''. Thus the change occurring in R in heterozygotes also is specific and depends on the particular allele in the homologous chromosome.

Tests have been made that exclude the cytoplasm as the basis of the phenomenon. Evidently, intrachromosomal changes at, or near, the R locus are involved. Definitive evidence concerning the stage in plant development at which the inherited alteration in R occurs is not yet available.

PAPAZIAN [9]: The interplay of nucleic and epinucleic will be involved when cells that are not "made for each other," in Stern's apt phrase, are crossed. A feature of dual control, nucleic and epinucleic, is that the nucleic is a long-term storage, controlling evolutionary change, whereas the epigenetic is short term.

A necessary requirement for the proper working of this system in evolution is that the epinucleic information be obliterated at each generation and be regenerated *de novo* from the nucleic information of the sperm and egg, cells that are, in this special sense, "made for each other." Only thus can long-term evolutionary change be under absolute and unadulterated control of the organ that is so efficiently designed for just that purpose, the nucleic apparatus.

Progeny from the cross of a liver and a kidney cell will contain a complex of nucleic and still-functional epinucleic combinations nice to unravel.

[9] H. Papazian, New Haven, Connecticut.

(Postscript, August 1958). The genetic basis of antibody formation was mentioned several times during the conference. An antigen may be thought to play either of two roles: *instructive* if the specifications for an antibody are introduced into the cell by an antigen, or *elective* if a preexistent synthesis is potentiated. [The terms "inductive" and "selective" connote a different issue, in population genetics rather than physiology. For example, there are good grounds for inferring an *elective* role of the substrate in enzyme *induction* (Lederberg, '56).]

It would be easier to choose between these roles if we knew more of the molecular basis of antibody specificity. If the γ-globulin molecules of a given animal have the same amino acid sequence, diverse antigens might plausibly instruct their folding in specific patterns (on the controversial assumption that sequence does not already predetermine folding). But how could the miscellany of antigenic substance convey instructions for different specific sequences of amino acids? An elective role for the antigen, on the other hand, would be equally compatible with any hypothesis of antibody structure.

The elective concept has been fully elaborated in Burnet's most recent proposals (Burnet, '57). He writes: "at some stage in embryonic development . . . a 'randomization' of the coding responsible for part of the specifications of gamma-globulin molecules, so that after several cell generations in early mesenchymal cells there are specifications in the genomes for virtually every variant that can exist as a gamma-globulin molecule. This must then be followed by a phase in which the randomly developed specification is stabilized and transferred as such to descendant cells." When a mature lymph cell preadapted to form a given antibody is stimulated by the corresponding antigen, it generates a larger clone of cells actively producing and liberating that antibody. Induced tolerance results from the hypersensitivity of embryonic cells so that prenatal experience of a given antigen abolishes the corresponding clone.

The least congenial feature of this hypothesis is doubtless that a separate clone must be maintained throughout the life

of the animal for each of the potential antibody responses. Whether these are numbered in thousands or billions is debatable, but in either case it is difficult to picture the maintenance of each clone against loss by random drift and especially against the selection for alternative species. To meet this and other objections the following revision was devised in the course of conversations with several participants at the conference, including Burnet. Its main departure is to propose that antibody-forming cells differentiate *throughout life* from a persistent stem line. As a corollary, randomization is a continuing process. The basis for tolerance remains hypersensitivity but of immature *cells* even in the adult animal.

This theme can be elaborated in several different ways. For one, close to Burnet's original, randomization would occur at a definite stage in histogenesis from the stem line. Diverse clones would be recurrently generated but need not survive indefinitely in the absence of the antigen. Hypersensitivity would likewise attend a definite stage of histogenesis. The introduction of an antigen from a time before any cells had matured past this stage would therefore suppress all homologous clones as they arose.

Alternatively, randomization might recur in the stem line itself, subject to stabilization and clonal expansion after a reaction with an antigen. The cell is immature immediately after its transition, hypersensitivity reflecting the reactions of minimal or early antibody.

Nothing has been said of the cytochemical locus of the randomization, nor of a number of other details of its genetic mechanism, such as the total number of possible states (= species of antibodies), their duration and multiplicity in a single cell, and their heritability before, during, and after antigenic stimulation. These and other items will have to be specified for detailed working hypotheses. Nor is tolerance necessarily founded on hypersensitivity, though this is the most plausible interpretation of the role of timing in the response to an antigen.

Current ideas on gene action are founded on the premise that the instructions for protein synthesis are filed in DNA and conveyed through RNA. On this premise, randomization would involve a particular segment of chromosomal DNA or microsomal RNA or both. In randomization, perhaps this patch is assembled at random from available nucleotides, rather than replicated in regular fashion. This mode of synthesis has already been suggested for heterochromatin, and likewise the aberration might be related to dissynchrony in nucleic acid synthesis. Less fanciful interpretations of hypermutability in certain metabolic states of the cell cannot yet be discounted.

The justification of this, as against other hypotheses, awaits experiments on the potentialities of clones derived from single adult cells. For the time being it rests on the proposition, so far uncontradicted, that tolerance can be maintained only in the continuous presence of the antigen, of which prenatally initiated chimerism is the perfect illustration. On Burnet's original version an antigen need suppress the homologous clone only during embryonic life and should be dispensable thereafter. The suggestion that stabilized clones are subject to remutations that must be dealt with to maintain tolerance is tantamount to, and in fact directly provoked, the present revision. Another expectation is that it should be possible to induce tolerance in populations derived from inocula small enough to preclude any cells already reactive to a given antigen. Experimentally, it may be necessary to excite the proliferation of such populations by other antigenic stimuli.

In previous discussion, I stressed the role of epinucleic effects largely for lack of a plausible nexus between embryonic inductions and nucleic information: how could nucleotide sequence be specifically altered by external, non-nucleic agencies? [See Nanney's discussion ('58) of epigenetic regulation, which has now appeared in detailed form.] The system of random hypermutability followed by elective stabilization, as proposed for antibody formation, furnishes another approach to this problem. Nucleic information could be

modified from without if it first underwent a series of random transitions, the apt one being recognized by the reactions of the corresponding products, and other inducers playing the part of antigens. Stabilization of the existing state is an instruction of a kind but far simpler than the predetermination of a nucleotide sequence. In particular, we need not invoke unprecedented reactions of DNA or RNA with external reagents beyond their already imputed functions in protein synthesis.

Antibody formation is the one form of cellular differentiation that inherently requires the utmost plasticity, a problem for which the hypermutability of a patch of DNA may be a specially evolved solution. Other aspects of differentiation may be more explicitly canalized under genotype control. If so, we might revert to the conception of local functional states of various genes whose specificity is unaltered. However, I can no longer insist that these states are epinucleic. Perhaps information that is nucleic but epigenetic should be dignified with another name, if and when it can be proved to exist either in microbiology or morphogenesis.

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